

# 139D NEUROBLASTOMA

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Neuroblastoma since its description in the late 19<sup>th</sup> and early 20<sup>th</sup> century has been well known as an enigmatic disease. Cushing and Wolbach described in 1927 the case of a 2-year old boy whose thoracic paravertebral sympathetic neuroblastoma over the course of 10 years transformed into a completely differentiated ganglioneuroma.<sup>1</sup> The only treatment at the time was injection of Coley's bacterial toxins. At the opposite end of the spectrum, the majority of neuroblastomas in older children present with widespread metastases and have a poor survival even with intensive chemotherapy and bone marrow transplantation (BMT). Our understanding of the basis for the diverse behavior of neuroblastoma has been greatly increased by our discovery of the molecular and genetic abnormalities in the tumors. This chapter reviews the biologic and clinical features of neuroblastoma, prognostic factors, and current therapeutic recommendations.

## EPIDEMIOLOGY

**INCIDENCE** In the United States, according to the SEER Program 1975–1995 monograph,<sup>2</sup> approximately 650 children and adolescents are diagnosed with neuroblastoma annually. Neuroblastoma accounts for 7.6% of all cancers in children less than 15 years of age, with the average age adjusted incidence rate of 9.1 per million children. However, there is a marked age dependence in the incidence rate, with an incidence rate for infants (less than 1 year) of 64 per million, dropping to 29 per million during the second year of life. Ninety percent of all cases occur before age 10 years. This predominance during infancy makes neuroblastoma the most common cancer occurring in the first year of life, with an incidence rate almost double that of leukemia, the next most common malignancy occurring in infants. The increased incidence rate among infants may be the result of the identification of previously undetected tumors in minimally symptomatic infants by noninvasive diagnostic tests or by the routine use of prenatal ultrasound.<sup>3,4</sup> These tumors if left undetected may have spontaneously regressed—a conclusion supported by the various urinary screening projects for neuroblastoma (see below).

Incidence rates were slightly higher in males (9.8 per million) than in females (9.2 per million), with the largest difference among infants (69.3 per million for males and 59.6 per million for females, respectively). There was also a racial difference between white and black infants but not in older children. The ratio of white to black incidence rates among infants was 1.7:1 for males and 1.9:1 for females.

**ENVIRONMENTAL RISK FACTORS** Despite the growing knowledge of the genetic changes that characterize neuroblastoma (see below), the cause of neuroblastoma has remained elusive. Given the young age at which most neuroblastomas present, it has been suggested that environmental exposures before conception or during pregnancy increase the risk of neuroblastoma. Epidemiologic investigations have implicated fetal exposure to diuretics, tranquilizers, hormones, phenytoin, alcohol, and tobacco as increasing the risk of neuroblastoma.<sup>5–8</sup> However, these studies have either lacked the statistical power to convincingly demonstrate that these drugs are etiologic risk factors or have failed to be confirmed by subsequent studies.

**PRENATAL DIAGNOSIS** The use of prenatal ultrasound has resulted in the detection of neuroblastoma in utero.<sup>3,4</sup> Infants diagnosed by prenatal ultrasound characteristically have lower stages tumors, favorable biologic features (*MYCN* nonamplified, hyperdiploid DNA index), and overall good prognosis with surgical resection often being curative.<sup>3,4</sup>

**SCREENING** Approximately 90 to 95% of patients with neuroblastoma secrete one or both of the urinary catecholamine metabolites, homovanillic acid (HVA; formed from dopamine) or vanillylmandelic acid (VMA; formed from norepinephrine). Screening of neuroblastoma by urinary VMA/HVA was initiated in the hope that earlier

detection of advanced disease would lead to more localized disease and thus increase the possibility of cure.<sup>9,10</sup> However, the cumulative data from 30 years of screening in Japan, the Quebec Neuroblastoma Screening (QNS) Project, and screening projects in Europe have shown that screening infants at 6 months of age or earlier results in a significant increase in the overall incidence of neuroblastoma but fails to reduce the incidence of advanced-stage patients with poor prognosis.<sup>11–14</sup> Neuroblastomas detected by screening have almost exclusively favorable biologic features (*MYCN* nonamplified, triploid, favorable Shimada histology).<sup>15,16</sup> Screening results in the overdiagnosis of tumors that would otherwise have spontaneously regressed. From the 1998 Consensus Conference on Neuroblastoma Screening held in Lyon,<sup>17</sup> it was concluded that routine screening in infants less than 7 months of age should be discontinued.<sup>18</sup> Whether screening at 12 or 18 months will be advantageous remains to be determined.

## MOLECULAR PATHOGENESIS

Cytogenetic and molecular abnormalities commonly found in neuroblastoma include whole chromosome gains, deletion of the short arm of chromosome 1, gain of genetic material on the long arm of chromosome 17, and genomic amplification of *MYCN* as seen by the presence of either homogeneous staining regions (HSRs) or double-minute chromatin bodies (dmns).<sup>19,20</sup> These genetic alterations strongly predict prognosis in neuroblastoma, with hyperdiploidy in infants correlating with favorable outcome, whereas deletion at 1p, gain at 17q, and amplification of *MYCN*, correlate with poor outcome. **GENETIC GAINS AND LOSSES DNA Index.** Flow cytometric analysis of the DNA content of neuroblastoma tumors has demonstrated that infants with a hyperdiploid DNA content (DI>1) are more likely to have lower stages of disease, improved response to chemotherapy, and have an overall improved outcome.<sup>21–24</sup> Cytogenetically, the hyperdiploid tumors found in infants frequently have whole chromosome gains with few structural rearrangements.<sup>23</sup> In contrast, tumors with a diploid DNA content (DI=1) or those hyperdiploid tumors of older children frequently have structural rearrangements such as translocations, deletions, or amplification. The favorable prognosis of hyperdiploid tumors appears to be restricted to infants and is currently used by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) to stratify treatment in infants with International Neuroblastoma Staging System (INSS) Stage 3, 4, and 4S disease (see below).

***MYCN* Amplification.** Genomic amplification of the proto-oncogene *MYCN* (also known as N-myc) is the result of amplification of a region from the distal short arm of chromosome 2 containing *MYCN* (2p24) onto dmns.<sup>19</sup> In a small percentage of neuroblastomas these dmns may then linearly integrate into random chromosomes as HSRs. Amplification may result in 50 to 400 copies of *MYCN*, with corresponding high levels of both *MYCN* mRNA and protein.<sup>25,26</sup> Amplification of *MYCN* appears to be an intrinsic genetic property of a tumor, with amplification consistently either present or absent in all tumor samples taken from an individual patient whether taken simultaneously or consecutively.<sup>27</sup>

Amplification of *MYCN* occurs in about 25% of patients with neuroblastoma, with amplification being a powerful predictor of advanced stage, rapidly progressing disease, and poor prognosis.<sup>23,28,29</sup> In an analysis of 3000 neuroblastoma tumors, only 4% of localized stage 1 and 2 tumors had *MYCN* amplification, and 8% of stage 4S patients, whereas 31% of advanced stage 3 and 4 patients had *MYCN* amplification.<sup>19</sup> In the small percentage of patients with lower stages of disease (stage 1, 2, or 4S), amplification may predict a poor outcome.<sup>19,30</sup> In a POG study of infants with stage D(S) (similar to INSS 4S) disease, infants with *MYCN* amplification had a significantly worse survival (44% versus 90%).<sup>31</sup>

Although numerous studies have clearly demonstrated that genomic amplification of *MYCN* is highly prognostic for poor outcome, analysis of the role of *MYCN* expression has been less clear. In particular, various studies have come to different conclusions regarding the role of *MYCN* expression in neuroblastoma in which *MYCN* is non-amplified.<sup>32</sup> Recently, however, Bordow and colleagues,<sup>33</sup> have

found that high *MYCN* gene expression is strongly predicative of outcome in older children with neuroblastoma but not in infants.

**Gain of 17q.** Gain of genetic material on chromosome 17 is the most common genetic abnormality in neuroblastoma.<sup>34,35</sup> Plantaz and colleagues,<sup>35</sup> demonstrated chromosome 17 gains by comparative genomic hybridization in 72% of cases, with 34% involving the whole chromosome and 38% the q arm alone, with the common region of gain 17q21.3-qter. Similarly, in a series of 313 patients, Bown and colleagues<sup>34</sup> have found unbalanced gain of 17q21-qter in 53.7% of tumors. The gain of 17q was characteristic of tumors in children greater than 1 year of age and advanced stage tumors, and was strongly associated with the deletion of 1p and amplification of *MYCN*. In multivariate analysis, gain of 17q was the most powerful prognostic factor, followed by the presence of stage 4 disease and deletion of 1p. The finding of recurring gains on chromosome 17 suggests the presence of one or more oncogenes on 17q that may contribute to neuroblastoma pathogenesis.

**Loss at 1p.** Deletion of the short arm of chromosome 1 is a common cytogenetic feature of neuroblastoma occurring in 30 to 50% of primary tumors. The common region of deletion or loss of heterozygosity (LOH) lies at the distal end of chromosome 1 in the area of 1p36.<sup>36</sup> This LOH suggests the presence of a tumor suppressor gene(s) within this region. Several candidate genes for the neuroblastoma suppressor gene have been mapped to 1p36; however, none have been shown to have mutations in the nondeleted allele.

Both 1p LOH and *MYCN* amplification correlate strongly with poor survival and with each other. Several studies have come to different conclusions as to whether 1p LOH and *MYCN* amplification are independent prognostic markers of outcome.<sup>37-40</sup> A recent study<sup>20</sup> has found that 1p LOH independently predicts for a decreased event-free (EFS) survival but not overall survival. They suggest that 1p LOH may predict for patients with localized disease who relapse but who can be salvaged with further therapy. Resolution of the independent prognostic value of 1p LOH versus *MYCN* amplification will most likely require identification of the tumor suppressor gene(s) located on 1p and elucidation of its function and relationship with *MYCN*.

**Loss at 11q and 14q.** Loss at 11q occurs frequently in neuroblastoma, with molecular studies demonstrating loss in 30 to 40% of tumors. The common region maps to the region of 11q23, suggesting the presence of a tumor suppressor gene in 11q. Analysis of 295 primary neuroblastomas has found, in contrast to 1p LOH, a significant inverse correlation with *MYCN* amplification.<sup>41</sup> When the *MYCN* unamplified patients were analyzed; those with 11q LOH had a significant decrease in overall survival. Thus, 11q LOH may help identify unfavorable patients who do not have *MYCN* amplification.

Deletion of the long arm of chromosome 14 is also a frequent occurrence in neuroblastoma, with the frequency around 25 to 50%. LOH for 14q correlates highly with 11q LOH and inversely with *MYCN* amplification.<sup>42</sup> It remains to be determined whether a tumor suppressor gene also resides in 14q.

**Familial Neuroblastoma.** About 1 to 2% of patients with neuroblastoma have a family history of the disease.<sup>43</sup> Familial neuroblastoma is inherited in an autosomal dominant Mendelian fashion with incomplete penetrance. Patients with familial neuroblastoma are usually diagnosed in infancy and can have multiple primary tumors, suggesting the presence of a germline mutation in one allele of a tumor suppressor gene.<sup>43,44</sup> Linkage analysis of families with neuroblastoma has excluded several regions including 1p36.<sup>45</sup>

**Neurotrophins.** Neuroblastoma is a tumor derived from the sympathoadrenal lineage of the neural crest. Since neurotrophins and their receptors have a critical role in the development of the nervous system, there has been considerable interest in their role in neuroblastoma pathogenesis. Members of the neurotrophin family include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), with NGF binding to TrkA, BDNF/NT-4 binding to TrkB, and NT-3 binding to TrkC.

Expression of high levels of TrkA correlates with lower stage and inversely with *MYCN* amplification.<sup>46-49</sup> Conversely, expression of TrkB correlates with *MYCN* amplified tumors.<sup>50</sup> Most of the TrkB

expressing tumors also express its ligand BDNF, which suggests that there may be an autocrine loop that promotes cell proliferation or survival.<sup>51</sup> TrkC is also expressed in neuroblastoma and correlates with favorable tumors that express TrkA.<sup>52,53</sup> The high expression of TrkA with or without TrkC may lead to either apoptosis in the absence of NT or to differentiation in the presence of NT.<sup>51</sup> The expression of Trk may explain the clinical observation that neuroblastomas can spontaneously regress or may differentiate into benign ganglioneuromas.

**Telomerase.** Telomerase is a ribonucleoprotein enzyme that maintains the integrity of ends of eukaryotic chromosomes, called telomeres. In contrast to most normal somatic cells, most human tumors express telomerase, resulting in stabilization of telomere length and permitting continued cell proliferation.<sup>54</sup> Telomerase expression is found in the vast majority of neuroblastoma tumors, with high telomerase activity correlating with advanced stage and improved outcome.<sup>55-57</sup> Due to the universal expression of telomerase in neuroblastoma tumors, and the finding of Hahn and colleagues,<sup>58</sup> that inhibition of telomerase limits the growth of human cancer cells, telomerase may be an attractive target for new antineoplastic therapies.

**Metastasis Related Genes.** *CD44* represents a heterogeneous group of cell surface glycoproteins that are involved with cell-cell and cell-matrix interactions. Their aberrant expression is associated with metastases and progression in various tumors, with lack of expression of *CD44* variants associated with advanced extrahepatic bile duct/ampullary carcinoma,<sup>59</sup> and tumor progression or recurrence in ovarian tumors<sup>60</sup> and bladder carcinoma.<sup>61</sup> The majority of neuroblastoma tumors express high levels of *CD44*, except for tumors of advanced stage and *MYCN* amplification.<sup>62-64</sup> Lack of *CD44* expression is strongly associated with *MYCN* amplification and in multivariate analysis is an independent predictor of overall survival.

The genes *nm23-H1* and *nm23-H2* encode proteins that have nucleoside diphosphate kinase activity and have been proposed to suppress metastases. In tumors such as breast cancer and melanoma reduced expression of *nm23* correlates with acquisition of metastatic behavior.<sup>65,66</sup> In contrast, in acute myeloid leukemia,<sup>67</sup> and non-Hodgkin's lymphoma,<sup>68</sup> increased expression of *nm23-H1* is associated with poor prognosis. In neuroblastoma, increased expression is associated with more advanced stages of disease and may be independently prognostic.<sup>69</sup> Genomic amplification of *nm23-H1* but not *nm23-H2* was found by Leone and colleagues<sup>70</sup> in 6 of 18 advanced-stage tumors. In addition, mutations have been identified in both *nm23-H2* and *nm23-H1*.<sup>70,71</sup> The precise role of *nm23* proteins in aggressive neuroblastoma remains to be elucidated.

Other factors likely to be involved in neuroblastoma metastases include the matrix metalloproteinases, integrins, and vascular endothelial growth factor (VEGF). The metalloproteinases *MMP2* (gelatinase A) and *MMP9* (gelatinase B) are expressed in primary neuroblastoma tumors, with increased expression associated with more advanced stage.<sup>72</sup> In addition, Ara and colleagues,<sup>73</sup> have demonstrated that decreased expression of the tissue inhibitor of metalloproteinase (TIMP-2) correlates with poor clinical outcome. Expression of the integrin  $\alpha$ V $\beta$ 3 has been associated with metastatic melanoma and breast cancer and may contribute to the malignant phenotype.<sup>74,75</sup> Integrin  $\alpha$ V $\beta$ 3 appears to be predominantly expressed in undifferentiated neuroblastoma,<sup>76</sup> with an antiangiogenic integrin  $\alpha$ V antagonist able to inhibit growth of neuroblastoma in a syngeneic murine tumor model by working synergistically with an anti-GD2 antibody.<sup>77</sup> The angiogenic factor VEGF has been found in both neuroblastoma cell lines and in primary tumors,<sup>78,79</sup> with an anti-VEGF antibody causing significant inhibition of neuroblastoma tumors in a nude mice model.<sup>80</sup> It remains to be determined whether new agents such as metalloproteinase inhibitors, integrin antagonists, or inhibitors of the VEGF pathway will be clinically useful.

## PROGNOSTIC MARKERS

**SERUM MARKERS** Neuroblastoma tumors produce numerous factors that can be measured in serum such as ferritin, neuron-specific enolase (NSE), chromogranin A, lactate dehydrogenase (LDH), and ganglioside G<sub>D2</sub>. Elevation of the serum ferritin (greater than 142 ng/ml) has been associated with poor outcome in neuroblastoma and when

combined with tumor histology can be highly prognostic.<sup>81–83</sup> NSE, although not specific for neuroblastoma, is associated with poor outcome in patients with advanced stage disease.<sup>84</sup> Similarly, serum levels of chromagranin A is associated with higher stage-disease and poorer outcome.<sup>85</sup> Serum LDH is predicative of outcome independent of both age and stage.<sup>86</sup> However, serum LDH does contribute additional information regarding risk groups that is not obtained from DNA ploidy, *MYCN*, or histopathology.<sup>87</sup> The ganglioside GD2 is expressed on the cell surface of the majority of neuroblastomas and can be used not only to identify neuroblastoma cells but its measurement in the serum may reflect either disease activity or response to treatment.<sup>88</sup> The importance of GD2 may not be due to its use as a prognostic marker but rather as a target for antibody therapy (see below).

**URINE MARKERS** About 90 to 95% of neuroblastomas produces catecholamines that can be detected as metabolites in urine. The predominant urinary catecholamines are (HVA) and (VMA). In addition to their usefulness in the diagnosis of neuroblastoma, the ratio of VMA/HVA has been a useful prognostic marker, with patients with a VMA/HVA ratio greater than 1 having improved survival.<sup>89,90</sup> In particular, in a study by Evans and colleagues, patients with stage III or IV disease with a VMA/HVA ratio greater than 1 had a 71% survival versus 17%.<sup>90</sup>

**TUMOR GENETIC MARKERS** As discussed in the section on molecular pathogenesis numerous genetic factors have been described that are of prognostic significance. These include DNA ploidy in infants, *MYCN* amplification, gain at 17q, 1p LOH, high TrkA expression, and *CD44*. Combination of these factors may help define subsets of neuroblastoma that may be more prone to relapse or progression. However, due to the known prognostic power and the ease with which DNA ploidy (by flow cytometry) and *MYCN* amplification (by fluorescent in situ hybridization) can be measured, these are only the tumor markers that are currently used to stratify patients into risk groups. As more is known about the prognostic value of gain at 17q, 1p LOH, TrkA, and *CD44*, it may be beneficial to incorporate these tumor markers into the next risk stratification schema.

**CLINICAL FACTORS** The two most important clinical factors that predict prognosis are the patient's age at diagnosis and stage of disease. Infants (1 year of age) have an overall improved survival when compared to older patients. In particular, infants with stage 3 or 4 disease have a remarkably good survival (90%) as long as their tumors do not have *MYCN* amplification.<sup>24,91,92</sup> Patients with stage 1 or 2 disease do well regardless of their age. Patients with primary tumors arising in the adrenal gland have a poorer prognosis than patients with tumors arising at other sites. However, location of the primary tumor does not appear to add significantly to prognosis above that supplied by age and stage. In an analysis of metastatic sites in 648 patients with stage IV or IVS disease, when adjusted for age, bone, intracranial or orbital, and lung sites were significantly unfavorable for infants and older children.<sup>93</sup> However, when multivariate analysis was performed these sites were not independent prognostic factors.

**MULTIVARIATE ANALYSIS OF RISK FACTORS** Given the wide number of risk factors described in neuroblastoma, there has not been a single study that has included all variables in a multivariate analysis. The factors that are consistently independently prognostic in numerous studies are age, stage, *MYCN* amplification, tumor histology, and ploidy in infants (see below). These factors are currently being used to stratify patients into risk groups (see below).

## DIAGNOSIS AND STAGING

**SIGNS AND SYMPTOMS** Neuroblastoma can originate from any site in the sympathetic nervous system, presenting as a mass in the abdomen, mediastinum, neck or pelvis. The most common primary site is the adrenal gland, with mediastinal tumors more common in infants than in older children (Table 139D.1). At presentation, about half of the patients will have metastatic disease, with the most common sites of metastases being bone marrow, bone, lymph node, and liver (Table 139D.2). Infants are much more likely to have liver metastases than children older than 1 year of age.

The signs and symptoms of neuroblastoma are dependent on the location and extent of the primary tumor, as well as the presence of metastatic disease. Large abdominal masses may cause complaints of

**Table 139D.1. Percent Distribution of Neuroblastoma by Primary Site and Age, SEER 1975–1995<sup>2</sup>**

Primary site	Age < 1 year	Age > 1 year
Adrenal gland	37	40
Connective, subcutaneous, soft tissue	21	16
Retroperitoneum	13	15
Mediastinum	13	6
Central nervous system	4	9
Autonomic nervous system	4	5
Other sites	8	10

fullness, discomfort, vomiting, or anorexia. Masses arising from the organ of Zuckerkandl in the pelvis can cause constipation and bladder dysfunction. High thoracic or cervical masses can present with unilateral ptosis, melosis, and anhidrosis (Horner syndrome). Epidural or intradural extension of neuroblastoma occurs in 5–16% of cases of neuroblastoma.<sup>94–97</sup> These patients may have symptoms associated with spinal cord compression including pain, bladder or bowel dysfunction, paraparesis or paraplegia. Prompt administration of chemotherapy appears to be an effective therapy for treating intraspinal neuroblastoma without the long-term sequelae associated with either radiation or surgical resection and laminectomy.<sup>94,98</sup>

Metastatic neuroblastoma can classically present with proptosis and periorbital ecchymoses and bone pain resulting in irritability and limp. Rapid enlargement of the liver metastases can result in respiratory compromise particularly in neonates.<sup>99</sup> Skin lesions, seen almost exclusively in infants, have a bluish hue and have been given the nickname “blueberry muffin” lesions.

**PARANEOPLASTIC SYNDROMES** The excessive secretion of catecholamines by tumors may very rarely lead to attacks of sweating, flushing, pallor, headaches, palpitation, and hypertension. Excretion of vasoactive intestinal polypeptide (VIP) can cause intractable watery diarrhea, with hypokalemia, dehydration, and failure to thrive.<sup>100</sup> VIP secretin tumors are histologically usually either ganglioneuroblastoma or ganglioneuroma and have favorable outcomes.<sup>101,102</sup>

Opsomyoclonus is a syndrome of bursts of rapid chaotic eye movements along with frequent, irregular, jerking movements of muscles that occurs infrequently in neuroblastoma patients. Overall patients with opsomyoclonus have a favorable outcome with respect to survival; however, many patients remain symptomatic even when no tumor is present. Unfortunately, many of these children have long-term neurologic deficits, including learning disabilities, motor and language delay, and behavioral abnormalities.<sup>103,104</sup> Treatment with adrenocorticotropic hormone or steroids has improved opsomyoclonus in some patients.<sup>102,104</sup>

**DIAGNOSTIC EVALUATION** The staging systems currently used in neuroblastoma are shown in Table 139D.3. In order to facilitate the

**Table 139D.2. Sites of Metastasis at Diagnosis for Patients with Stage IVS (n = 81), Stage IV < 1 year (n = 133), and Stage IV > 1 year (n = 434) by Percent<sup>93</sup>**

Site	Stage IVS	Stage IV < 1 yr	Stage IV > 1 yr	Total
Bone marrow	35	57	81	70
Bone	0	49	68	56
Lymph node	9	29	36	31
Liver	80	53	13	30
Intracranial/Orbit	0	26	20	18
Adrenal	6	13	6	8
Skin	14	8	1	4
Pleura	0	4	4	3
Lung	0	2	4	3
Peritoneum	0	4	2	2
Other	0	4	2	2
Central nervous system	0	0	1	1

comparison of clinical trials and biologic studies, an international consensus regarding the criteria for the diagnosis of neuroblastoma, staging system, and response criteria was published in 1988<sup>105</sup> and revised in 1993.<sup>106</sup> The staging system is termed the INSS, shown in Table 139D.3, and the response criteria the International Neuroblastoma Response Criteria (INRC), shown in Table 139D.4. A diagnosis of neuroblastoma is established either by unequivocal tumor histopathology or an elevated VMA and/or HVA along with unequivocal tumor cells in the bone marrow. If tumor histology is equivocal, then genetic features such as *MYCN* amplification or 1p LOH can be used to support the diagnosis of neuroblastoma.

To assess for extent of disease, computerized tomography and/or magnetic resonance imaging scan is recommended to evaluate the primary tumor and liver or lymph node metastases. For bone marrow metastases, bilateral bone marrow aspirates and biopsies are required. For cortical bone involvement, the best test is a metaiodobenzylguanidine (MIBG) scintigraphy or a <sup>99</sup>Tc bone scan if an MIBG scan is unavailable or negative. In infants, a skeletal survey should be performed since it is generally a more reliable indicator of bone disease than a <sup>99</sup>Tc bone scan.

**PATHOLOGY** The histopathologic appearance of neuroblastoma ranges from undifferentiated neuroblasts, to more mature ganglioneuroblastoma, to fully differentiated and benign ganglioneuroma. Several pathologic systems have been developed as prognostic guides, including those developed by Shimada and colleagues and Joshi and colleagues.<sup>107–110</sup> Recently, a morphological classification system based on the system proposed by Shimada and colleagues was recommended by the International Neuroblastoma Pathology Committee (INPC).<sup>111,112</sup> The INPC classification is dependent on age, the degree of differentiation grade of the neuroblasts, the cellular turnover index, and the presence or absence of Schwannian stromal development. The four categories and their subtypes are: neuroblastoma (Schwannian stroma poor), undifferentiated, poorly differentiated, and differentiating; ganglioneuroblastoma, intermixed (Schwannian stroma rich); ganglioneuroma (Schwannian stroma dominant); and ganglioneuroblastoma, nodular (composite Schwannian stroma rich/stroma dominant and stroma poor). Favorable histology groups, regardless of age, are ganglioneuromas and ganglioneuroblastomas (intermixed). Favorable neuroblastomas with age < 18 months have poorly differentiated or differentiating and low or intermediate mitotic karyorrhectic index (MKI) tumors, and age 18 months to 5 years have differentiating and low MKI tumors. Ganglioneuroblastoma with nodular appearance is an unfavorable histology independent of age. Unfavorable histology neuroblastomas with age < 18 months have

**Table 139D.3A. International Neuroblastoma Staging System<sup>105,106</sup>**

Stage	Definition
1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically
2A	Localized tumor with incomplete gross excision; representative ipsilateral lymph nodes negative for tumor microscopically
2B	Localized tumor with or without complete gross excision, with ipsilateral lymph nodes positive for tumor, enlarged contralateral lymph nodes must be negative microscopically
3	Unresectable unilateral tumor infiltrating across the midline, with or without regional lymph node involvement; localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement
4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, and other organs (except as defined for stage 4S)
4S	Localized primary tumor (as defined as stage 1, 2A, or 2B), in patient <1 year, with dissemination limited to skin, liver, and/or bone marrow (marrow involvement should be minimal with malignant cells <10% of total nucleated cells)

**Table 139D.3B. Evans/CCG Staging System<sup>232</sup>**

Stage	Definition
I	Tumor confined to the organ or structure of origin
II	Tumor extending in continuity beyond the organ or structure of origin, but not crossing the midline; regional lymph nodes on the ipsilateral side possibly involved
III	Tumor extending in continuity beyond the midline. Regional lymph nodes may be involved bilaterally
IV	Remote disease involving the bone, bone marrow, soft tissue, or distant lymph node groups
IV-S	As defined by Stage I or II, except for the presence of remote disease confined to the liver, skin, or marrow, without bone metastases

undifferentiated tumors or high MKI, and with age 18 months to 5 years have undifferentiated or poorly differentiated tumors or intermediate or high MKI tumors. Patient with neuroblastoma with age greater than 5 years have unfavorable histology.

**STAGING SYSTEMS** Prior to implementation of the INSS,<sup>105,106</sup> the two staging systems that were in use in the United States were the system described by Evans and colleagues<sup>113</sup> (Table 139D.B) and the system described by St. Jude and later modified by the POG<sup>114</sup> (Table 139D.C). Like the St. Jude system, the INSS takes into account the extent of tumor resection (Table 139D.A: INSS stages 1A, 2A, 2B, and 3). The INSS also retains the “special” category of 4S for infants (less than 1 year) with a localized primary tumor who have metastases to the liver, skin, and minimal amount in bone marrow (10%). Infants with stage 4S disease have an overall excellent prognosis, with tumors that often undergo spontaneous regression.<sup>115</sup>

## TREATMENT

**RISK GROUPS AND TREATMENT ASSIGNMENT** Working risk groups with suggested treatment assignment based on recent cooperative clinical trials have been developed by the collaborative efforts of the CCG and the POG. The risk assignment depends on age, INSS stage, *MYCN* gene copy number, histopathology, and, for infants, tumor cell DNA ploidy (Table 139D.5). Survival by INSS stage is shown in Plate 45, Fig. 139D.1.

**LOW RISK** Low-risk patients are comprised of those with localized tumors (INSS 1 and most patients with INSS 2 disease) and infants with 4S disease who have favorable tumor biology. These patients have an excellent survival with a primary surgical approach and supportive care. Children with INSS 1 and 2 have now been shown to have 5-year survival of 95% with a primary surgical approach.<sup>116–120</sup> Chemotherapy is reserved only for the small numbers of patients with symptomatic disease due to paraspinal tumors resulting in spinal cord compression. Even patients who have some local recurrence of tumor after surgery are usually easily salvageable with either further surgery or with moderate chemotherapy or local radiation. Neither radiation nor

**Table 139D.3C. St. Jude/Pediatric Oncology Group Staging System**

Stage	Definition
A	Complete gross resection of primary tumor, with or without microscopic residual disease. Intracavitary lymph nodes not adherent to the primary tumor are histologically negative. Nodes adhered to the surface or within the primary tumor can be positive. Liver histologically free of tumor
B	Grossly unresected primary tumor. Lymph nodes are the same as in stage A. Liver histologically free of tumor
C	Complete or incomplete resection of primary tumor. Intracavitary lymph nodes not adhered to the primary tumor are histologically positive. Liver histologically free of tumor
D	Dissemination of disease beyond intracavitary nodes (i.e., distant lymph nodes, bone, bone marrow, liver, or skin)
DS	Infants <1 year of age with stage IV-S disease (see Children's Cancer Group)

Response	Primary Tumor	Metastatic Sites
CR	No tumor	No tumor; catecholamines normal
VGPR	Decreased by 90–99%	No tumor; catecholamines normal; residual <sup>99</sup> Tc bone changes allowed
PR	Decreased by >50%	All measurable sites decreased by >50%. Bones and bone marrow: number of positive bone sites decreased by >50%, no more than 1 positive bone marrow site allowed
MR	No new lesions; >50% reduction of any measurable lesion with with <50% reduction in any other; <25% increase in any existing lesion	
NR	No new lesions; <50% reduction but <25% increase in any existing lesion	
PD	Any new lesion; increase of any measurable lesion by >25%; previous negative marrow positive for tumor	

CR = complete response; VGPR = very good partial response; PR = partial response; MR = mixed response; NR = no response; PD = progressive disease.

chemotherapy has been shown to contribute to survival in this group as a whole.<sup>117,121,122</sup> The exceptions are children over 1 year at diagnosis with tumor *MYCN* amplification, who usually show rapid tumor progression and dissemination with this management and may benefit from very aggressive treatment.<sup>29</sup> Other biologic features, such as Shimada unfavorable histology,<sup>109,119</sup> ploidy,<sup>23</sup> or significant amounts of occult bone marrow tumor by immunocytology,<sup>123</sup> may also define the rare patient who will progress, but this needs validation in a very large prospective study due to the rarity of unfavorable biologic features in this group.

Patients with stage 4S with favorable biologic features also have an excellent survival of 85 to 90% with minimal therapy.<sup>31,124–127</sup> Treatment has ranged from supportive care only to modest chemotherapy regimens given over 4 to 5 months. Age less than 2 months at diagnosis, *MYCN* amplification, unfavorable Shimada histopathology and diploidy have been prognostic factors identified as significant for either decreased survival or decreased EFS in recent studies.<sup>31,126</sup>

However, approximately 40 to 50% of infants do require some cytotoxic therapy due to symptomatology. The patients who die from 4S neuroblastoma are generally those who present with very extensive hepatic infiltration, causing respiratory impairment and occasionally partial renal and venous obstruction. These children may require emergency abdominal decompression, mechanical ventilation, and chemotherapy and hepatic radiotherapy, which can result in sepsis and other complications leading to death.

Most of these are very young infants with favorable biologic features, suggesting that ultimate survival would have been excellent if the symptomatology could have been more quickly relieved. This raises the therapeutic dilemma in these infants of who deserves treatment and what type of treatment. Late effects of chemotherapy and radiation in the very young infant may be more significant than in older children, and it is difficult to justify putting an entire population at risk for the 50% who require more than supportive care.

Based on the consistent data from multiple groups that the majority of deaths are in children under 2 months, this group should be treated at the very first symptoms. A scoring system has been proposed that could be used and evaluated prospectively, based on emesis, respiratory compromise, venous return, renal function, and thrombocytopenia. Patients less than 1 month had twice the symptoms of older infants. Each system is scored as 1 or 2, and any total of 2 points mandates treatment.<sup>99</sup> Current treatment recommended is low-dose chemotherapy and, in cases of imminent organ failure, cross-table low-dose hepatic radiation. However, this may not be sufficient to rescue the severely compromised infant, and also puts infants at risk from late complications of radiation at a young age.

**INTERMEDIATE RISK** The intermediate risk-group is comprised of patients who have an excellent prognosis, with 80 to 90% survival, including stage 3 with favorable tumor biology, stage 4 infants without tumor *MYCN* amplification, and stage 4S patients whose tumors are diploid or have unfavorable histopathology. These results have been achieved with surgery, local radiation therapy for residual unresectable tumor, and moderately intensive combination chemotherapy. This group includes the patients of any age with biologically favorable stage 3 tumors, defined by the absence of *MYCN* amplification, favorable Shimada classification, and serum ferritin 143 ng/ml.<sup>91</sup> Patients with stage 3 disease who are less than 1 year at diagnosis and have a single copy of *MYCN*, regardless of other biologic features, also are included because of their favorable outcome.<sup>91</sup> Infants less than 1 year at diagnosis with stage 4 disease whose tumors have a single copy of *MYCN* have a disease-free survival in excess of 85% with conventional chemotherapy, regardless of other prognostic features.<sup>24,92,93</sup> The other group of intermediate-risk patients are infants with stage 4s disease who have either diploid DNA index or unfavorable Shimada histopathologic classification.<sup>24,126</sup> Currently, an intergroup CCG POG study is underway to determine whether the excellent prognosis of the above group can be maintained with only 4 cycles of chemotherapy given over 12 weeks, using a combination of carboplatin, etoposide, doxorubicin, and cyclophosphamide. Patients with less favorable biology, including infants with either unfavorable histology or diploidy, will receive 8 rather than 4 cycles of therapy.

**HIGH RISK** The high-risk group in neuroblastoma is comprised primarily of patients with stage 4 disease greater than 1 year at diagnosis but also includes stage 3 with either *MYCN* amplification or those greater than 1 year with unfavorable Shimada, stage 2 greater than 1 year with *MYCN* amplification, and stages 3, and 4 infants with *MYCN* gene amplification. Despite the use of increasingly aggressive combined modality treatments, which have increased remission rate and duration, the long-term survival for INSS stage 4 disease in children who are greater than 1 year of age at diagnosis has remained, until recently, less than 15%. More recently, the introduction of platinum-based therapy, the use of increasingly dose-intensive chemotherapy combinations and the incorporation of myeloablative therapy followed by treatment for minimal residual disease has resulted in improvement in rates of progression-free survival and overall survival with 3-year estimates at 30%. The 4-year survival for all stage 4 patients greater than 1 year at diagnosis in the CCG studies from 1978 to 1985 (n = 507) was 9%, as compared to 30% for patients treated from 1991 to 1995 (n 675) (P 0.001).<sup>129</sup>

Current optimal therapy includes a chemotherapy induction phase, incorporation of local control with surgery and radiotherapy for bulky disease and myeloablative conditioning supported by hematopoietic

Table 139D.5. Risk Groups for Treatment

INSS	Age (days)	<i>MYCN</i>	Histology	Ploidy	Risk
1	Any	Any	Any	Any	Low
2A/2B	< 365	Any	Any	Any	Low
	≥ 365	Nonamplified	Any	—	Low
	≥ 365	Amplified	FH	—	Low
	≥ 365	Amplified	UH	—	High
3	< 365	Nonamplified	Any	Any	Intermediate
	< 365	Amplified	Any	Any	High
	≥ 365	Nonamplified	FH	—	Intermediate
	≥ 365	Nonamplified	UH	—	High
	≥ 365	Nonamplified	Any	—	High
4	< 365	Nonamplified	Any	Any	Intermediate
	< 365	Amplified	Any	Any	High
	≥ 365	Amplified	Any	—	High
4S	< 365	Nonamplified	FH	DI > 1	Low
	< 365	Nonamplified	FH	DI = 1	Intermediate
	< 365	Nonamplified	UH	Any	Intermediate
	< 365	Nonamplified	Any	Any	High
	< 365	Amplified	Any	Any	High

stem cell reconstitution, followed by therapy for minimal residual disease. The function of induction therapy is maximal reduction of tumor burden, including *in vivo* purging of bone marrow tumor, within a time frame, which will minimize the risk of developing resistant tumor clones and clinical progression. Effective *in vivo* purging of bone marrow and peripheral blood tumor is essential if autologous hematopoietic stem cell rescue is to be the source of marrow reconstitution following marrow ablative therapy. Induction therapy usually incorporates radiotherapy and surgery for local tumor control although the definitive proof of the value of total body irradiation (TBI),<sup>130</sup> local radiation,<sup>91,131–134</sup> or aggressive local surgery<sup>128, 135–137</sup> remains controversial in the absence of a prospective randomized study.

Interpretation and comparison of response rates in different studies is fraught with difficulty, due to differing response criteria. There is now international agreement to use the International Response Criteria see (Table 139D.4) in order to compare results. However, the use of the sensitive detection method of 123 I- or 131 I-MIBG scans<sup>138,140</sup> or fluorodeoxyglucose positron emission tomography (PET)<sup>140</sup> may significantly influence interpretation of disease response, since either of these modalities may be more sensitive and specific than the more traditional technetium bone scans and CT scans for detection of small amounts of disease. Additionally, due to the sporadic nature of bone marrow involvement in this disease, both the number of sites for bone marrow aspirates and biopsies and the use of the more sensitive immunocytology may influence interpretation of disease response.<sup>123,141–144</sup>

The most effective induction regimens for obtaining complete or partial response are combination platinum-based regimens including a combination of other active drugs, such as cyclophosphamide, doxorubicin, etoposide, vincristine, and ifosfamide. Induction regimens used in recent large cooperative studies are shown in Table 139D.6, with overall response rates (CR PR) ranging from about 60 to 90% at the end of 5 to 6 months of treatment. Most of these also include surgery to residual disease, although the overall impact of complete

**Table 139D.6. Induction Regimens for High-Risk Neuroblastoma since 1985 (> 50 patients)**

Group/ Reference	Year	Regimen*	N	CR + PR(%)
POG 8742 (regimen 1) <sup>145</sup>	1987–91	day 1–5, CDDP 40mg/m <sup>2</sup> /day; day 2–4, VP16 100 mg/m <sup>2</sup> /day. This alternates q 21 days with day 1–7, CPM PO 150 mg/m <sup>2</sup> /day; day 8, DOX 35mg/m <sup>2</sup> .	111	77
POG 8742 (regimen 2) <sup>145</sup>	1987–91	day 1, CDDP 90mg/m <sup>2</sup> ; day 2, VP16 100 mg/m <sup>2</sup> ; day 3–10, CPM 150 mg/m <sup>2</sup> /day P.O.; day 11, DOX 35mg/m <sup>2</sup> Repeat q 21 days.	115	68
SFOP CADO/PE <sup>233</sup>	1987-92	day 1–5, CPM 300 mg/m <sup>2</sup> /d day 1 & 5, VCR1.5 mg/m <sup>2</sup> /d day 5, DOX 60 mg/m <sup>2</sup> /d Alternates q 21 days for 2 cycles each with day 1–5, CDDP 40 mg/m <sup>2</sup> /d day 1–5, VP16 100 mg/m <sup>2</sup> /d	183	64
Study Group of Japan <sup>234</sup>	1985-97	day 1, CPM 1200 mg/m <sup>2</sup> , VCR 1.5 mg/m <sup>2</sup> day 3, THP-ADR 40 mg/m <sup>2</sup> day 5, CDDP 90 mg/m <sup>2</sup> Repeat q 28 days x 6 cycles	168	92
CCG 3891 <sup>149</sup>	1991-96	day 1, CDDP 60/m <sup>2</sup> ; day 3, DOX 30 mg/m <sup>2</sup> ; day 3 & 6, VP16 100 mg/m <sup>2</sup> /day; day 4 & 5, CPM 900 mg/m <sup>2</sup> /day ; Repeat q 28 days x 5 cycles	539	78

POG = Pediatric Oncology Group; CCG = Children's Cancer Group.

resection on survival in stage 4 disease is still contradictory, as noted above. Some newer single agents have also been shown to have efficacy in newly diagnosed neuroblastoma, using the “up-front phase II window” approach. Following two courses of single-agent therapy prior to induction treatment, response rates (CRPR) for effective agents 30% response were easily detectable, including ifosfamide, carboplatin, iproplatin,<sup>145</sup> and topotecan.<sup>146</sup> Two agents that were less effective in this setting were epirubicin<sup>145</sup> and paclitaxel.<sup>146</sup> There was no evidence that such a design adversely affected the subsequent outcome of patients when compared to the outcome of patients treated with similar induction without the phase II window.

**MYELOABLATIVE THERAPY WITH HEMATOPOIETIC STEM CELL SUPPORT** Myeloablative high-dose chemotherapy with or without (TBI) has been incorporated as consolidation treatment for high-risk neuroblastoma for the past two decades, beginning with early studies using melphalan ablation. Numerous single-arm studies employing various myeloablative regimens between 1985 to about 1995 were completed and showed an apparent improvement in EFS, usually reported from time of transplantation. The 3-year EFS ranged from 26 to 62%, with an average of about 40% for the 11 large studies (greater than 30 patients) shown in Table 139D.7.<sup>147,154</sup> This approach introduced some bias into the interpretation of results, since only patients who survived for 5 to 6 months and responded to induction chemotherapy were able to receive the high-dose myeloablative treatment with autologous or allogeneic transplantation.

Several retrospective comparisons of concomitant and similar groups of patients treated with standard doses of chemotherapy versus those treated with high-dose therapy and autologous bone marrow transplantation (ABMT) have been performed, with conflicting assessments of the value of myeloablative therapy. The CCG treated children with high-risk neuroblastoma with either monthly cycles of 4-drug chemotherapy over 1 year or, at investigator discretion, with myeloablative therapy with cisplatin, etoposide, melphalan and TBI followed by purged ABMT. By the end of 5 to 7 courses of chemotherapy, 159 patients were event-free, and 67 of these went on to ABMT, whereas 74 continued chemotherapy for a total of 1 year. In a retrospective, nonrandomized comparison of ABMT versus traditional chemotherapy, EFS of patients who were progression-free at 8 months from diagnosis was significantly superior for patients receiving ABMT (40% vs. 19%). An even more significant advantage for ABMT was seen for those patients who were only in partial, as opposed to complete, remission at the end of induction and for those

**Table 139D.7. Autologous Bone Marrow Transplantation Conditioning Regimens and EFS from Time of ABMT for Neuroblastoma in First Response (Trials with > 30 Patients)**

Reference	Regimen	N	3-year EFS (%)
235	Melphalan, TBI	62	38
152, 153	1. Cisplatin, VM-26, Doxorubicin, melphalan, TBI	45	42
	2. Cisplatin, VP-16, melphalan, TBI	54	50
	3. Carboplatin, VP-16, melphalan, TBI	48	41
236	Carmustine, teniposide, melphalan (total of one or two courses)	33 15 (1 course) 18 (2 courses)	49 (2-year EFS)
151	Vincristine, melphalan, TBI	62	30
150	Etoposide, melphalan or cisplatin, etoposide, THP-Adriamycin, melphalan, with or without TBI	31	50 (5-year EFS)
147	Vincristine, melphalan, TBI	34	29
148	European Bone Marrow Registry Data	549	26 (5-year EFS)
149	Carboplatin, VP-16, melphalan, TBI	129	43
154	Carboplatin, VP-16, melphalan, local radiation	77	62

EFS = event-free survival; TBI = total body irradiation.

who with tumor *MYCN* amplification.<sup>153</sup> These results were in agreement with results from the Lyon group, in which Philip and co-workers showed a difference in 2-year survival of 39% versus 12% for concomitant patients treated either with myeloablative therapy and ABMT compared to standard chemotherapy.<sup>155</sup> In contrast, a retrospective comparison from the POG of 116 patients achieving complete or partial remission did not show any significant difference in outcome for those undergoing BMT prior to progression.<sup>156</sup>

In order to eliminate the bias inherent in such retrospective comparisons, a randomized prospective trial was necessary. Pinkerton reported the first attempt at a randomized trial of high-dose therapy with autologous bone marrow support for neuroblastoma, with a suggestion of improvement noted for ABMT. This report however, included a total of only 65 randomized patients, with widely varying times to ABMT.<sup>157</sup> The first large prospective, randomized study comparing a single course of myeloablative chemoradiotherapy supported by purged ABMT to three cycles of a dose-intensive, nonmyeloablative, continuous infusion, consolidation chemotherapy was conducted by the CCG. Of 379 eligible patients who were randomized, 85% had INSS stage 4 tumors. Randomized patients were assigned equally to ABMT (n = 189) or consolidation chemotherapy (n = 190), whereas 118 were nonrandomly assigned to consolidation chemotherapy due to parental or investigator refusal. Overall toxicity and total mean hospital days were similar between the two randomized arms. The 3-year EFS was 27% for all patients, 34 ± 4% for those assigned to ABMT, and 22 ± 4% for those cases randomly assigned to consolidation chemotherapy, (P 0.034)(Fig. 139D.2). Overall survival was not significantly different between the two regimens, perhaps partly due to use of ABMT salvage in 33 of the patients who progressed after consolidation chemotherapy. The outcome advantage noted with ABMT was also significant in the subgroups of patients with *MYCN*-amplified tumors and in those who were greater than 2 years of age at diagnosis. The comparison of outcome from the time of BMT to other regimens is given in Table 139D.7. Here, in order to compare the EFS of the randomized study to previous studies, where EFS was measured from the time of transplant, an “as-treated” analysis rather than an “intent-to-treat” analysis is shown. The 3-year EFS from time of transplantation for those 129 patients actually receiving ABMT as-treated analysis is shown to be 43 ± 6%, similar to previous single-arm studies and significantly higher than the 26 ± 5% EFS of the 150 patients actually receiving the chemotherapy consolidation.<sup>149</sup>

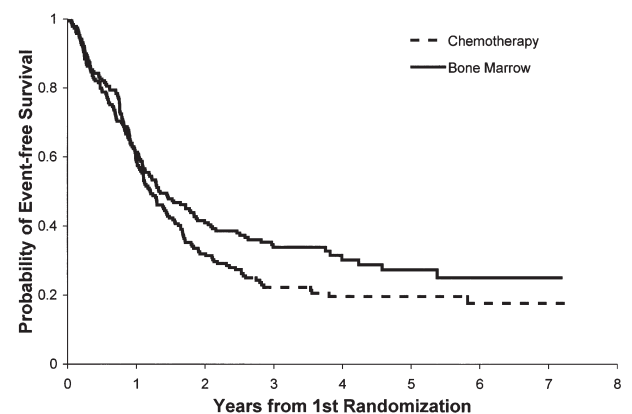
Despite the advantage ABMT appears to have over conventional therapy in high-risk patients, caution must be used in interpreting these data. Follow-up remains relatively short, and there remains the risk of late recurrences, as noted in the French experience.<sup>157</sup> Nevertheless, it is clear that aggressive induction therapy followed by some form of myeloablative therapy and stem cell re-infusion has provided a superior window of minimal residual disease.

Many clinical and biologic risk factors have been studied using either single-study or pooled data, which potentially affect the outcome after BMT. Multiple myeloablative conditioning regimens have been tested, with no apparent difference in EFS as seen in the examples in Table 139D.7. The CCG tested 3 consecutive conditioning regimens in the 321P3 study, given after five cycles of a similar induction with cisplatin, etoposide, doxorubicin, and cyclophosphamide. After local control with surgery and radiation to residual disease sites, patients then received ablative chemotherapy and TBI with purged autologous transplantation. No significant difference was seen in the 3 year EFS with the three different regimens.<sup>152,153</sup> An analysis of 509 patients by Dini and colleagues for the European bone Marrow Transplant Registry similarly was unable to detect an effect of particular conditioning regimen on outcome.<sup>130</sup> Despite possible theoretical advantages, allogeneic BMT was not superior to autologous purged BMT in multiple comparative reports and was associated with higher toxicity and death rate.<sup>158–160</sup> The most significant factor that appears to adversely influence EFS in patients undergoing BMT is detection of residual disease at the time of myeloablative therapy,<sup>149</sup> assessed either by conventional methods, bone marrow immunocytology,<sup>161</sup> or MIBG scan.<sup>148</sup> Interestingly, within the group of patients undergoing ABMT, *MYCN* amplification has not been prognostic, although it is a prognostic factor for patients receiving standard chemotherapy.

**BONE MARROW PURGING** Bone marrow involvement by neuroblastoma is extremely common in children with metastatic disease, present by light microscopy in 60 to 80% of children with INSS stage 4 disease at diagnosis.<sup>93</sup> Detection of tumor by immunocytology using a mixture of monoclonal antibodies reactive at the cell surface has recently been shown to reliably detect tumor with a sensitivity that may vary from 1 to 10 per 10<sup>5</sup> nucleated bone marrow cells, depending on the method used.<sup>123,143,162–166</sup> Evaluation of the efficacy of *in vivo* purging of tumor cells in bone marrow or blood may be critical for evaluation of hematopoietic stem cell products, since the use of myeloablative therapy followed by autologous hematopoietic stem cell transplantation has been shown to be beneficial to EFS.<sup>149</sup> Furthermore, since bone marrow or peripheral hematopoietic stem cells are necessary to reconstitute patients after myeloablative tumor therapy, an induction regimen capable of efficient tumor cell reduction in bone marrow may be important. The ability of re-infused occult tumor cells in bone marrow to cause relapse is demonstrated by the report that after infusion of unpurged autologous bone marrow marked with transduced neomycin-resistance gene, tumor cells in the recurrent neuroblastoma in all three cases showed the genetic marker.<sup>167</sup> The occasional reports of miliary lung relapse after BMT, the site one would expect to be involved after intravenous infusion of tumor cells, supports the importance of tumor-free stem cells.<sup>168</sup>

Circulating tumor cells can also be detected in the blood of up to 50% of children with INSS stage 4 neuroblastoma at diagnosis.<sup>161,163</sup> This test, with a sensitivity of 1 tumor cell per 100,000 nucleated cells, demonstrates that although marked reduction occurs in both bone marrow tumor and circulating tumor cells, contamination of bone marrow can be detected in 25% of bone marrow samples at the end of 3 months of induction chemotherapy and in 7% of blood samples.<sup>161</sup> The efficacy of *in vivo* purging has been shown to correlate with EFS, such that patients with more than 0.1% tumor in bone marrow at the end of induction have a very poor outcome. Measurement of residual tumor cells in both blood and bone marrow by immunocytology and by the possibly more sensitive technique of RT-PCR<sup>169,170</sup> may prove a useful surrogate marker of response and detect patients with more resistant disease.

**NEW MYELOABLATIVE REGIMENS** One current approach to further improve the success of myeloablative therapy for neuroblastoma is by further intensifying the pre-ABMT conditioning regimen. One way this can be accomplished without increasing the toxicity is by elimination of TBI, thereby allowing higher local irradiation doses and higher chemotherapy doses. Preliminary data in 77 high-risk patients in first response using this modified protocol showed a 3-year EFS of 62 ± 20%. Toxic deaths were only 4%, with no toxic deaths in those patients with good renal function (GFR > 100 ml/min).<sup>154</sup> This condi-



**Figure 139D.2.** Event-free survival from time of first randomization (8 weeks from diagnosis) for patients randomized to transplantation (n = 189) versus chemotherapy consolidation (n = 190); 3-year event-free survival P = 0.034.<sup>149</sup>

tioning regimen will be used in the next U.S. cooperative study for high-risk neuroblastoma.

Other ways to intensify therapy without unacceptable toxicity include tumor-specific agents or repetitive myeloablative regimens with hematopoietic stem cell support. Targeted radioisotopes, such as  $^{131}\text{I}$ -MIBG<sup>171,172</sup> or  $^{131}\text{I}$ -anti-GD2 antibody<sup>173</sup> are being investigated both alone, or in combination with chemotherapy as myeloablative regimens. Agents that enhance the activity of a chemotherapeutic agent are also under investigation, such as the combination of buthionine sulfoximine with melphalan. Glutathione (GSH) is a tri-peptide that protects cells from oxidative stress, binds to and detoxifies nucleophilic drugs, and may play a role in chemo-resistance.<sup>174</sup> Buthionine sulfoximine (BSO) inhibits GSH synthesis by inhibition of the rate limiting- synthetic enzyme (gamma glutamylcysteine synthetase) and has been shown to reduce GSH levels and enhance tumor response to both radiation and chemotherapy *in vitro*.<sup>175</sup> A pilot study of BSO with melphalan in children with relapsed neuroblastoma showed responses in 20% of patients and tolerable toxicity.<sup>176</sup> A dose escalation study of the melphalan with BSO followed by peripheral blood stem cells is underway.

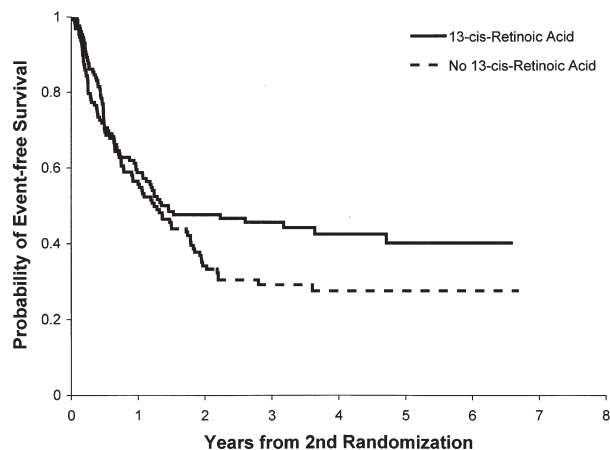
Efforts are also underway to intensify the transplant conditioning by administering more than one ablative regimen with multiple stem cell infusions. One of the earlier double transplant regimens was a pilot study in 33 children with metastatic neuroblastoma reported by Philip and colleagues.<sup>177</sup> Children received induction therapy with etoposide and cisplatin or carboplatin, then megatherapy with teniposide, carmustine, and cisplatin with autologous purged bone marrow support. They underwent a second harvest 2 to 3 months after the first ABMT, then a second conditioning with vincristine, melphalan and TBI. The toxic death rate was markedly increased after the second graft (7/30) and engraftment was delayed.<sup>177</sup> More recent studies testing double or triple myeloablative therapy have therefore uses peripheral blood stem cells rather than bone marrow in order to obtain adequate cells prior to megatherapy and to obtain more rapid engraftment. Diller and co-workers have recently reported preliminary results of double transplant in neuroblastoma, uses two successive myeloablative regimens (etoposide, carboplatin, and cyclophosphamide, then melphalan with (TBI) each supported by peripheral blood stem cell infusion. Of 21 eligible patients, 16 completed both first and second transplants, with initial promising EFS of 68% (CI 46–90%).<sup>178</sup> A cooperative group efficacy trial is currently underway in the POG to evaluate this technique in a multiinstitutional setting. Thirty-three patients are enrolled and 18 have completed the two transplants with no transplant-related deaths. Outcome results are pending. A triple ABMT pilot is being conducted at Memorial Children's Hospital in Chicago by Kletzel and colleagues. Induction therapy uses combination therapy with cisplatin, etoposide, cyclophosphamide, doxorubicin, and ifosfamide, with the addition of two courses of anti-GD<sup>2</sup> and three courses of high-dose cyclophosphamide followed by peripheral blood stem cell harvest. Conditioning regimen includes carboplatin and etoposide for the first two procedures and thiotepa with cyclophosphamide for the third. Currently, 20 patients are enrolled with 15, 13 and 10 having completed the first, second, and third transplants, respectively. Thirteen patients remain disease-free 25 to 43 months from diagnosis and 4 to 36 months following the last transplant procedure. It remains to be determined if either of these strategies will impact outcome.

**TREATMENT FOR MINIMAL RESIDUAL DISEASE** Despite this progress, many children with high-risk disease relapse after myeloablative therapy, even when they appear to have achieved complete remission. Although in the past relapse has tended to occur in the primary site or other bulky disease metastases,<sup>134,179</sup> in more recent years, relapse is frequently distant, usually involving the bone and bone marrow.<sup>93</sup> With improved surgical techniques and higher dose local irradiation, relapse in the primary may become less common.<sup>133,154</sup> Relapse after intensive therapy implies a high degree of resistance to standard cytotoxic drugs, confirmed by *in vitro* studies,<sup>180</sup> and mandates a different type of approach to eradication of malignant cells, such as targeted therapy or use of tumor differentiating agents.

Immediately post-ABMT, when disease is likely to be minimal, provides the ideal window of time to eradicate resistant clones that are still present with the introduction of novel therapies not dependent upon standard cytotoxic mechanisms. *In vitro*, both all-trans retinoic acid and 13-cis-retinoic acid cause decreased proliferation and differentiation in neuroblastoma cell lines, including some established from refractory tumors.<sup>181–184</sup> A phase I trial in children with high-risk neuroblastoma determined that a high-dose intermittent schedule of 13 cis-retinoic acid following BMT had minimal toxicity, achieved levels that were effective against neuroblastoma cell lines *in vitro*, and resulted in complete bone marrow responses in 3 of 10 patients.<sup>185</sup> These data indicated that 13 cis-retinoic acid is well tolerated after intensive chemoradiotherapy and may have efficacy against minimal residual disease. A subsequent phase III randomized trial by the CCG of children with high-risk neuroblastoma completing consolidation chemotherapy or ABMT showed that the use of oral 13 cis-retinoic acid following intensive therapy improves outcome. The 3-year EFS from time of randomization was significantly better for the patients randomized to 13 cis-retinoic acid ( $46 \pm 6\%$ , compared to those randomized to no further therapy ( $29 \pm 5\%$  percent;  $P\ 0.03$ )(Figure 139D.3).<sup>149</sup>

Other retinoids may further enhance the ability to eradicate minimal disease. A synthetic retinoid, fenretinide (N-(4-hydroxyphenyl) retinamide; 4-HPR), has been reported to inhibit the growth of neuroblastoma.<sup>186–188</sup> Its action is independent of the cell cycle, which suggests that fenretinide may be active in malignant cells resistant to chemotherapy by this mechanism. Importantly, fenretinide is effective against cell lines known to be resistant to cis- or transretinoic acid, including a cell line derived from a patient who had relapsed after receiving 13-cisretinoic acid post-ABMT.<sup>189,190</sup> In contrast to cis- and trans-retinoic acid, fenretinide does not induce maturational changes but is cytotoxic and induces both apoptosis and necrosis.<sup>189</sup> A striking degree of apoptosis, measured by TdT labeling of DNA fragments with flow cytometry, was noted as early as 36 hours following exposure to fenretinide in the 13-cisretinoic acid resistant LHN neuroblastoma cell line.<sup>191</sup> Therefore, fenretinide may be active in patients with neuroblastoma resistant to myeloablative doses of chemoradiotherapy, as well as retinoic acid. A phase I study of fenretinide in refractory neuroblastoma has nearly been completed in the CHG,<sup>192</sup> and a phase II study is currently proposed.

Targeted therapy in relapsed neuroblastoma using murine, chimeric, and humanized antibodies against the membrane ganglioside GD<sup>2</sup> has provided promising response and toxicity profiles that warrant the further investigation of these agents in randomized studies. Therapeutic responses have been obtained in phase I and phase II studies of a murine IgG<sup>3</sup> monoclonal antibody, 3F8,<sup>173,193–195</sup> murine 14.G2a,<sup>196,197</sup> and human-mouse chimeric monoclonal antibody,



**Figure 139D.3.** Event-free survival from time of second randomization (34 weeks from diagnosis) for patients randomized to 13 cisretinoic acid (n = 130) versus no 13-cis-retinoic acid (n = 128); 3-year event-free survival  $P = 0.027$ .<sup>149</sup>

ch14.18.<sup>198–201</sup> With GM-CSF or IL-2, anti-GD<sup>2</sup> seems to be tolerated in patients who have undergone ABMT.<sup>196,202</sup> A new randomized prospective trial of the use of chimeric anti-GD2 antibody with GM-CSF and IL-2 will be undertaken as a national cooperative endeavor in advanced neuroblastoma after stem cell transplant.

**TREATMENT OF RELAPSE** Despite intensive therapies as above, relapse continues to be a problem in approximately half of patients who achieve initial remission. Recent studies in cell lines obtained from patients at diagnosis and relapse suggest the development of various resistant phenotypes in heavily treated patients, including resistance to alkylating agents such as melphalan and to platinum compounds, doxorubicin, and etoposide.<sup>180</sup>

Multiple single-agent and intensive combination regimens have been tested in refractory and relapsed patients. High-dose carboplatin and cyclophosphamide elicited responses in 6 of 11 evaluable patients, with a high rate of hematologic toxicity.<sup>203</sup> Combined continuous infusion cisplatin, doxorubicin, and etoposide with daily bolus ifosfamide resulted in responses in 15 of 40 children with refractory neuroblastoma, although again with a high rate of mucositis and of hematologic toxicity, including toxic death in 2.5%.<sup>204</sup> High-dose platinum with thiosulfate and etoposide, n = 5, resulted in unacceptable renal toxicity, whereas high-dose carboplatin and etoposide achieved 10 of 22 responses with 80% grade 3 and 4 hematologic toxicity; ifosfamide and carboplatin showed 9 of 23 responses and hematological toxicity in 50%.<sup>205</sup> Topotecan alone and in combination with cyclophosphamide has yielded responses in refractory neuroblastoma in phase I studies.<sup>206–208</sup> In phase II studies, the response rate for neuroblastoma has been higher on the daily bolus 5 day schedule than on a 72 hour continuous infusion, where only 1 of 26 patients with neuroblastoma responded.<sup>209,210</sup> A current phase II randomized POG-CCG study is ongoing, comparing topotecan with topotecan and cyclophosphamide in a randomized trial. However, a word of caution is necessary, since *in vitro* studies of neuroblastoma cell lines suggest that lines that are resistant to topoisomerase II inhibitors, such as etoposide, are also resistant to topoisomerase I inhibitors, such as topotecan. Thus, it is not clear yet what topotecan adds to the treatment of relapse patients.<sup>211</sup> The mode of administration may also affect susceptibility to drugs. Thus, even in patients who have relapsed after regimens containing intravenous etoposide, responses are common to oral etoposide.<sup>212</sup> The role of myeloablative therapy for relapse remains somewhat controversial. In most reported studies, EFS has ranged from only 10 to 20%.<sup>130,154,158,213</sup>

Targeted radioisotope therapy using antibody or MIBG for delivery of radiation in the form of <sup>131</sup>Iodine has also been tested extensively in clinical trials in relapsed neuroblastoma. Cheung and co-workers have reported on the use of <sup>131</sup>I-3F8 for treatment of refractory neuroblastoma with documented responses and now have an ongoing study for newly diagnosed patients using <sup>131</sup>I-3F8 in myeloablative doses followed by bone marrow rescue, then further treatment with cold antibody post-transplant.<sup>173,214</sup> <sup>131</sup>I-MIBG has been widely shown in European and American studies to elicit about a 30% response rate for refractory neuroblastoma.<sup>215,220</sup> More recently, it has also been effective for initial therapy in patients with regional disease.<sup>221,222</sup> A phase I dose escalation trial of <sup>131</sup>I-MIBG reported by Matthay and colleagues determined the maximal nonmarrow nonablative dose (12mCi/kg) and the maximal practical ablative dose with stem cell rescue (18 mCi/kg). In 30 patients, there was a 37% response rate and no significant toxicity other than hematologic.<sup>217</sup> Future studies will be testing the combination of <sup>131</sup>I-MIBG with ablative chemotherapy for resistant neuroblastoma.

Other tumor-specific approaches under investigation that show some promise in the laboratory and in preliminary phase I and II studies include tumor-differentiating agents, immunologic modulators, including tumor-specific antibody, antibody given with GM-CSF or IL-2, fusion proteins of antibody with cytokines, and tumor vaccines, uses neuroblastoma cells transduced with various immunomodulators or anti-idiotypic vaccines.<sup>196,223–230</sup>

**ADOLESCENT AND ADULT NEUROBLASTOMA** Neuroblastoma after age 10 is extremely rare, accounting for less than 10% of cases. The course of the disease in older children and adults tends to be much

more indolent than in the younger children, with recurrences and waxing and waning of disease occurring over as long a period as 20 years. Despite this indolent course, the long-term survival is poor. In a single institution series of 16 patients over age 12 reported by Franks and colleagues, only one survivor remained, although 7 of the patients had local or regional disease only.<sup>231</sup> The actuarial survival of all CCG patients ages 13 to 18 years was 7% at 5 years and 4% at 10 years, whereas that for patients ages 1 to 13 years was 30% at 5 years and 23% at 10 years.<sup>231</sup> It is possible that the course may differ from the disease in younger children due to differing biologic characteristics. Tumors are rarely *MYCN* amplified in older patients, as shown by a series of 32 patients in the CCG over age 12, of whom only 1 patient had *MYCN* amplification. Catecholamine secretion is also reported to be less frequent in older patients.<sup>231</sup> Because of the poor outcome with local therapy, it has been suggested that adolescent and adult patients with neuroblastoma should be treated more aggressively with cytotoxic therapy even for low-stage disease, such as INSS 1 and 2, as well as treating those with stages 3 and 4 intensively.

## CONCLUSIONS AND FUTURE PLANS

In conclusion, substantial advances are occurring in the definition of the molecular pathogenesis of neuroblastoma, as well as in better definition of clinical prognostic groups. Modest but significant improvements have also been achieved in the treatment of metastatic disease by increasing dose intensity with the use of hematopoietic support. However, new therapeutic approaches are required using tumor targeting, differentiating or apoptotic agents, stimulation of host immune response, or genetic manipulation in order to overcome chemotherapy resistance.

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